bicarbonate (R_{60Bic}) was defined as the difference in the decline of % PI- and Fluo-3 negative sperm within 60 min of incubation in medium A and B ($R_{60Bic} = \Delta_{A3-A60} - \Delta_{B3-B60}$). Despite considerable variability, there were neither significant correlations between the specific response to bicarbonate (range: 3.4–69.3%) and the percentage of total or progressive motile sperm (70.9–98.4% and 42.4–96.7%, respectively), nor with the percentage of PI- and FITC-PNA-negative sperm (39.8–98.3%; $p > 0.05$). However, a negative correlation existed between the specific response to bicarbonate (R_{60Bic}) and the number of morphological abnormal sperm per sample (1.5–54.0%; $r = -0.72$; $p < 0.001$). Sperm with cytoplasmic droplets (0.5–37.0% per sample) accounted for the most frequent observed defect (47.3%) of all abnormal sperm. Correlation of R_{60Bic} was negative with respect to this specific defect ($r = -0.68$; $p < 0.001$). In conclusion, semen samples with higher incidence of sperm with cytoplasmic droplets show a reduced responsiveness to bicarbonate, whereas other sperm parameters are not related to the bicarbonate response. As retained cytoplasmic droplets are considered as a sign of immaturity, incomplete sperm maturation seems to be associated with impaired membrane function affecting the signalling cascade of the capacitation process.

OC 10

Expression analysis of porcine aromatase (CYP19) as a specific target gene in testis

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Cytochrome P450 aromatase is the key enzyme in estrogen biosynthesis, encoded by the *CYP19* gene. However, little is know about the *CYP19* roles in boar spermatogenesis. Therefore, the aim of this work was to investigate the mRNA and protein expression of CYP19 in boar reproductive tissues from boars with different sperm quality. For mRNA and protein expression study, a total of six boars were divided into two groups with Group I (G-I) and Group II (G-II), where G-I is characterized for a relatively better sperm quality. For the expression study between reproductive and non-reproductive tissues by semi-quantitative PCR study, mRNA from all six boars was pooled together according to the tissues. On the other hand, mRNA and protein expression study in different reproductive tissues from two divergent groups of animals were performed by semi-quantitative PCR, qRT-PCR and western blot, respectively. Due to the limitations of fresh samples from G-I and G-II boars, different fresh testis from a healthy breeding boar was collected after slaughtering for protein localization by immunofluorescence. The remarkable *CYP19* mRNA expression was detected only in testis. The mRNA expression of *CYP19* was not detectable in other reproductive tissue (epididymis and accessory glands) and non-reproductive tissue (brain, liver and muscle) by semi-quantitative PCR. When mRNA expression in reproductive tissues from G-I and G-II boars was compared by semi-quantitative PCR, the *CYP19* was detectable in testis for G-I and G-II boars. However, mRNA and protein expression were not differentially regulated between G-I and G-II boars ($p > 0.05$). The CYP19 protein was detected in testis from G-I and G-II boars. This protein expression result of western blot appeared to be consistent with results of the qRT-PCR. The CYP19 protein localization observed a strong staining in the cytoplasm of Leydig cells in testis. The mRNA and protein expression study of the CYP19 imply that it might have a role in spermatogenesis in pigs, which is specifically expressed in boar testis. However, association study and more functional study in boars with extreme divergent phenotypes are required.

OC 11

Characteristics of boar semen stored at different temperatures

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Purpose: To evaluate storage and temperature effects on extended boar semen characteristics and fertility outcomes. **Methods:** Extended semen (143 ejaculates/76 boars) were examined within 1 day of collection and after 4 days storage at 20.4 ± 0.1°C (RT) or 14.7 ± 0.2°C (CT). Fertility data (total, live, mummified, stillborn pigs) was derived from 535 inseminations (sow parity range 1–8) using same-batch semen (41/76 boars) stored at 17°C for 0–4 days at stud. Evaluations included CASA (IVOS) sperm motility (total, progressive, rapid, VCL, VAP, VSL, BCF), sperm clump score (0–3), semen pH and temperature. Data were analysed by one-way ANOVA, GLM (stillbirths) and linear regressions (total born and born live) plus chi-square analysis for sow returns and farrowings. Inseminations were performed across four seasons.
Results: Initial (receiving) values were: concentration 49.3 ± 1.4 × 10^6/ml; temperature 18.7 ± 0.2°C; clump score 1.4 ± 0.05; pH 7.45 ± 0.02; motilities: total 57.7 ± 2.4%; progressive 29.3 ± 1.6%; rapid 43.9 ± 2.3%; VCL 154.4 ± 2.8; VAP 75.5 ± 1.6; VSL 43.5 ± 0.9; BCF 33.8 ± 0.3. Both RT and CT storage for 4 days resulted in a rise in pH (7.7 ± 0.02; $p < 0.01$) and similar ($p < 0.01$) declines in a number of motility parameters (total, progressive, rapid and BCF) as well as for VSL ($p < 0.05$). For CT, declines also occurred in VCL and VAP (both $p < 0.01$). Clump score was not influenced by storage time or temperature. The proportion of sows farrowing vs. returns was similar for semen stored for 1, 2 or 3+ days. However, storage time and season interacted with litter size ($p < 0.05$), as did storage time, dam line, and parity with % stillborn (both $p < 0.01$). Total sperm motility positively influenced % live pigs and both total and progressive motility negatively influenced % stillborn (all $p < 0.05$).
Conclusion: Storage of extended semen for 4 days at approximately 15 or 20°C resulted in similar decreases in CASA sperm motility assessments. Overall fertility was not influenced by storage time (0–4 days at 17°C). Despite this, sperm motility measures (total and progressive) influenced % live pigs and % stillborn and storage time interacted with both season and sow factors re. litter size and % stillborn. This research was made possible by financial assistance from Pork CRC Australia.